A Century of DNA

A History of the Discovery
of the Structure and Function
of the Genetic Substance

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7 Genetic Transformation by DNA

While geneticists focused on the mechanisms of heredity, they left to others the elucidation of the nature of the molecules responsible for these mechanisms. The possibility that the nucleic acid component of nuclein was the active genetic component was actually considered during the early 1900s.[1] Subsequently, the prevalent view was that only proteins fulfilled the requirements expected of the genetic material.[2] Quite unpredictably, the discovery of the molecular basis of heredity was accomplished through the study of the transformation of bacteria from one distinct genetic type to another.

The early work on bacterial transformation derived from studies of bacterial cultures. It had been found that bacteria could be grown only in fluid containing certain nutrients. In 1897, R. Kraus reported that certain bacterial species released specific precipitable substances into this fluid medium.[3] In 1907, the observation was extended to the specific class of bacteria termed *pneumococci.*[4] The early interest in pneumococcus derived from the fact that it caused lobar pneumonia, which at the time was a leading cause of death in the United States.

Initial observations by J. A. Arkwright at the Lister Institute in London in the early 1920s led to the characterization of two forms of certain bacteria. The virulent form was generally covered with a smooth coat (encapsulated) and hence was termed the *S form,* while the nonvirulent (nonencapsulated or attenuated) form was rougher and more irregular in shape and was termed the *R form* (figure 7.1).[5] In addition, studies over many years showed the existence of various distinct types of virulent S forms. For pneumococci, four types were initially delineated, designated I-IV, each of which produced characteristic immunological reactions. In patients with pneumonia, several types of S forms were often found, and their proportions frequently changed during the course of the infection.

In 1917, Oswald T. Avery (figure 7.2) and A. R. Dochez, working at the hospital of the Rockefeller Institute, demonstrated the presence of a "specific soluble substance" in the blood and urine of patients infected with pneumonia.[6] The germinal idea for these studies came from the earlier observation that bacteria secreted specific substances into the growth medium.[7] Thus, Avery and Dochez reasoned that such secreted materials would be found in the body fluids and might be involved in the disease. They found that the substance released was specific for the particular type of pneumococcus producing the infec-

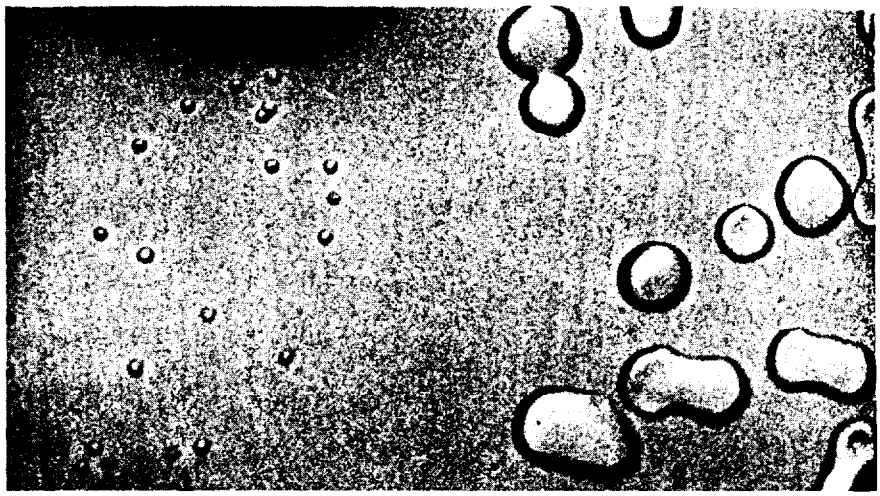

Figure 7.1 Rough (left) and smooth (right) colonies of pneumococcus (courtesy of *Journal of Experimental Medicine 79,* 137-158 [1944], plate 1).

tion. These investigations were pursued with the aim of understanding the specific basis of pneumonia as a disease and of developing methods of diagnosis and treatment. The presence of specific soluble substances in body fluids had, in fact, been reported eleven years earlier by W. Fornet for another disease, typhoid fever, but others had had difficulty in confirming this work.[8]

Certain bacteria such as diphtheria and tetanus were known to be pathogenic. Their effects were produced, however, not simply from their mere physical presence in the organism. Rather, they were known to secrete substances, termed *toxins,* into the surrounding fluids. It was the biological effects of these toxins that led to the disease and subsequent death. Thus, inoculation with the toxin alone produced the same effects as inoculation with the intact bacterium. In the case of pneumococcus, Avery and Dochez's discovery of a specific soluble substance suggested a situation similar to that of diphtheria. Yet further testing showed that the specific soluble substance probably did not have sufficient toxicity to account for the pathological effects produced by the bacterium. These authors also described the intravenous inoculation into rabbits of pneumococcus extracts that elicited a specific antibody response and the continued excretion into the urine of the specific soluble substance for several days—all in the absence of living pneumococci. There followed many

Figure 7.2 Oswald T. Avery (courtesy of the U.S. National Academy of Sciences).

detailed and elegant studies by Avery with Michael Heidelberger on the nature of these substances, studies that are now considered classical experiments in the science of immunology.[9]

The questions regarding immunological specificity were important ones. That bacterial infections caused animals to produce specific blood proteins, termed *antibodies,* had been recently established. These antibodies would react with the bacteria that produced them to form precipitates. It was this kind of reaction that enabled bacteriologists to distinguish between the different types of pneumococci described above. Thus, Type I pneumococcus would react with Type I blood or serum (the fluid remaining after removal of cells from blood) but not with Type II serum. Bacteriologists were intrigued by this phenomenon and recognized the diagnostic value it presented. This reaction was true for many kinds of bacterial infection. The important question that remained unanswered was, What chemical component or components making up the bacterium are required to produce the immunological specificity? It was the search for answers to this question that revealed a relationship between bacterial infection and the biological activity of DNA.

Hans Zinsser and Julia Parker, working at the department of bacteriology at Columbia University, reported in 1923 on the immune responses produced by different components of the bacteria causing tuberculosis, as measured by skin sensitivity. "In the work with tuberculosis although the residue antigen gave excellent skin reactions, somewhat stronger reactions had always been obtained by using the nucleoprotein fraction and it had never been possible to free this nucleoprotein fraction of its skin-reactive properties, however frequently it was precipitated and redissolved. The relationship between the nucleoprotein fraction and the residue is not clear . . . one possible explanation may be the fact that the nucleoprotein fraction represents the mother substance of the residue."[10] Thus, at the outset, nucleoprotein was implicated in effecting the immune response.

Avery and his associates sought to identify the active antigenic components of pneumococcus. In 1925, they found that the "specific soluble substance" of pneumococcus was a carbohydrate, which failed, however, to induce antibodies when injected alone into mice. But, as with the tubercle bacilli, the nucleoprotein fraction of pneumococcus was antigenic, that is, it induced antibody formation when inoculated.[11] They also showed that each pneumococcal type pro-

duced a distinct carbohydrate during infection, which was related to the composition of the smooth coat of the S form in each case.[12] Furthermore, the different pneumococcal types were recognized as being genetically distinct.

In 1928, Frederick Griffith (figure 7.3), a medical officer of the Ministry of Health in England, reported a startling discovery: he had observed genetic transformation of bacteria. Born at Hale in Cheshire in 1881, Griffith was an immensely dedicated scientist—a virtual recluse known to only a few close associates. His dedication cost him his life. During the blitz of London in 1941, Griffith was killed while working in his laboratory. "Fred Griffith was a modest and retiring personality who enjoyed working quietly on his own, shunning scientific meetings."[13] According to his colleague V. D. Allison, he practically had to be forced into a taxi to attend the London International Microbiology Congress in 1936. "And then, I am told, he reluctantly and nervously read out his rather boring paper in such an unenthusiastic manner that those not closely associated with the detailed streptococcal Typing techniques he was expounding must hardly have felt it was worth listening to. This was, it seems, the only paper he ever delivered at an open meeting and had, of course, nothing to do with transformation."[13]

As a microbiologist in a public health laboratory, Griffith had noted that several different types of pneumococci could be isolated from the sputum of individual patients suffering from pneumonia. Griffith's curiosity was aroused by the observation that over several years the incidence of one type of pneumococcus had markedly increased while another had decreased for patients in the Smethwick area of England. In attempting to find a basis for these changes, he carried out experiments in his laboratory to determine what factors might be involved in converting one type of pneumococcus to another type within the body of the host. Griffith observed that if he inoculated mice with a nonvirulent R form and a heat-killed S form, a transformation to the virulent S form was obtained quite readily. These transformations depended on the R and S types used. For example, "the most certain method of procuring reversion is by the inoculation of the R culture, subcutaneously into a mouse, together with a large dose of virulent culture of the same Type killed by heat."[14]

Another important observation was that "the inoculation into the subcutaneous tissues of mice of an attenuated R strain derived from

Figure 7.3 Frederick Griffith. This may be the only surviving photograph of Griffith, a copy of which was kept by Avery on his desk. (Courtesy of A. F. Coburn.)

one Type, together with a large dose of virulent culture of another Type killed by heating to 60°C, has resulted in the formation of a virulent S pneumococcus of the same Type as that of the heated culture."[14] Of course, the controls with each type injected separately rarely gave a virulent infection, since the heating of bacteria to 60° killed them, so that they would not be expected to show any biological activity. This observation of the transformation of pneumococcal type proved to be of the utmost significance.

Griffith at this time was in his fifties, was well established, and was known for his care in experimentation.[13] Nevertheless, it appears unlikely that he recognized the significance of his discovery of transformation. He failed to recognize the factor that the heat-killed S form was supplying and thought it was the "S substance . . . that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate. This protein seems to be necessary as material which enables the R form to build up the specific protein structure of the S form."[14] M. R. Pollock noted in reviewing this period, "Fred Griffith never hinted in his paper that he contemplated that nucleic acid might have had a part to play in his transformation. But it would have been quite logical and possible for him to have done so, and 'nucleoproteins' were in fact discussed in his laboratory in relation to the phenomenon."[13] His observations were very quickly confirmed. Indeed Hobart Reimann, working at the Rockefeller-financed Union Medical College in Peking, China, and F. Neufeld and W. Levinthal at the Robert Koch Institute in Berlin submitted their respective papers in the same year, 1928.[15,16]

Naturally there was interest in this phenomenon in Avery's laboratory, although "for many months Avery refused to accept the validity of this claim."[17] There were reasons for this: "It seemed at first to Avery a contradiction of the Type stability he had painstakingly demonstrated, but Neufeld's quick confirmation is said to have convinced him."[2] Martin Dawson, a Canadian working in the laboratory at the Rockefeller, carried out confirmatory experiments alone and published his findings without Avery as a coauthor.[18] This resulted partly because Avery was absent for some time due to illness but also partly from the "scepticism . . . understandable in one who had devoted so much effort and skill to the doctrine of immunological specificity."[17]

In 1929, Dawson moved to the College of Physicians and Surgeons

of Columbia University, and with Richard Sia, on leave from the Peking Union Medical College, continued his investigations. In his earlier paper, Dawson had described experiments in which he attempted to reproduce transformation in a bacterial culture as well as in a live animal. All his attempts to do so, and those of his predecessor Thomas Francis, Jr., had failed.[19] He had concluded that "either the conditions employed were unsuitable or living tissues were necessary for the transformation process."[20] However, by utilizing several different approaches, they were able eventually to successfully demonstrate transformation in culture with suspensions of heat-killed pneumococci, termed *vaccines.*[20] The success of such studies often depended on apparently minor technical considerations. For example, "The culture medium employed appears to be a matter of considerable importance. In all successful experiments either serum or red blood cells were added to the culture media. When plain broth alone was used transformation failed to occur. The suggestion is offered that the addition of the blood and serum may have afforded a convenient source of catalase and peroxidase. In the absence of these substances sufficiently reduced conditions may not have been present."[20]

This represented the second important breakthrough after Griffith's discovery of transformation because now it was possible to study the effects of various agents on the transformation process under controlled laboratory conditions. One of these conditions, and indeed one of the limitations of their technique, was the temperature sensitivity that they observed. The longer they heated the vaccine, or the higher the temperature they used, the lower was the ability to produce transformation in vitro. They attributed this to "thermolability and susceptibility to enzymes liberated in old broth cultures."[21] They also concluded that "the factor contained in an S vaccine responsible for transformation of Type is closely related to, if not identical with, the 'antigenic specific substance' of pneumococcus."[21]

A further significant step in the process of identifying the nature of the substance (or substances) responsible for transformation was made by Lionel Alloway, also in Avery's laboratory, who used extracts completely free of pneumococcal cells. "Alloway, who first produced a cell-free active transforming extract, was by all accounts more of a quiet, lone worker, but working in the same room with Avery, it is beyond question that he was, like all of us, spurred, stimulated and advised at many major turns in the road by his 'Professor.'"[2]

Alternate freezing and thawing caused the cells to rupture (lysis), and centrifugation removed the cell debris. After heating and filtering, the degree of transformation produced in R cells by this extract was just as high as that for the S type of pneumococcus.[22] By utilizing sodium desoxycholate, a detergent known to disrupt cells, and precipitation of the extract with alcohol, Alloway was subsequently able to obtain much more efficient extraction of the active substance. On adding alcohol he observed that "a thick stringy precipitate formed, which slowly settled out on standing."[23] How reminiscent of Miescher's preparations of nuclein! He concluded, like Dawson and Sia before him, that "the exact nature of the active material in these extracts still remains to be determined," although he was inclined to the view that it was protein (the type-specific antigen).[23]

Although Dawson and Alloway had worked in his laboratory, Avery's name did not appear on their publications as coauthor or in any acknowledgments. This was typical of his unassuming nature.[24,25] Recovered from several bouts of illness and convinced of the fact of transformation by the evidence, Avery now began to take an active part in the work devoted to characterization of the active transforming principle in the extracts.

By 1935, Avery was a well-established scientist with a strong reputation in the field of bacteriology. He had been born in Nova Scotia in 1877 but lived most of his life in New York City, where his father was a clergyman who undertook missionary work in the Bowery.[17] He took his M.D. at Columbia University in 1904 and joined the Rockefeller Institute in 1913. He remained there until 1948, when he retired to Nashville, Tennessee, where he died in 1955.[24,25] He was a bachelor and spent long hours in the laboratory, extraordinary for his age at the time of this work. From his professorial demeanor he was given the nickname "Fess." He was an extremely private person with a very gracious exterior, but he also had a "brooding forehead" and could be "a melancholy figure whistling gently to himself."[17] René Dubos, who was seeking a position at the Rockefeller Institute at the time, recalls, "Dr. Avery was a man of immense charm who always appeared to be interested in what *you* were interested in, but in fact, always managed to turn the conversation around to what *he* was interested in."[26] Dubos further recalls, "He was also very picturesque. 'We have a substance here,' he said as he opened his drawer and took out a tube. 'In this tube is the substance that makes up the capsule

that surrounds the pneumococcus. It's called Type 3 polysaccharide. If we only knew a way of decomposing that substance, that capsule, that polysaccharide that surrounds the pneumococcus, it would certainly open up all sorts of avenues.' "[26] The similarity in the painstaking approaches of Griffith and Avery to their work, their lack of undue speculation, their retiring characters, and how important these characteristics were to the difficult type of work in which they were engaged has been noted.[13] Yet the two never met.

Avery did not labor alone; he was joined from 1934 to 1941 by Colin MacLeod (figure 7.4) and, in 1942, by Maclyn McCarty (figure 7.5), a pediatrician and later a lieutenant in the naval reserve.[27] Before leaving, MacLeod refined the assay for the transforming agent so that the purification of the material could be repeated more readily. In the Rockefeller *Scientific Reports* for 1941-1942 Avery noted, "In the case of pneumococci, virulence becomes manifest only when the cells are encapsulated, that is the so-called smooth (S) phase. Pneumococci which are devoid of capsules, that is the so-called rough (R) phase, are avirulent even in the most susceptible animal species. It seems obvious that the presence of the capsule is intimately associated with the manifestation of the property of virulence. However, there is reason to think that the capsule is not alone the basic factor responsible for this property."[28] Avery enumerated a number of reasons for this conclusion. First, certain types, such as Type III, showed variable amounts of virulence although they had the same capsule. Thus, for two strains of Type III having equal virulence in mice, one was highly virulent in rabbits while the other showed no virulence. Second, there were cases where repeated rapid passage through a susceptible host increased the virulence of the type without a corresponding alteration in the chemical constitution of the capsular polysaccharide. Third, for Type I it was established that encapsulation was necessary for maximal virulence but when the organism was grown in the presence of a drug, sulfathiazole, virulence was lost while the capsular polysaccharide was retained. Thus, although the capsule was necessary for virulence, a direct correlation was not always evident.[28] The studies on the relationship between the carbohydrate capsule and virulence encouraged them to continue. These studies clearly suggested "that some of the basic factors responsible for virulence reside in the cell body and are unrelated to the capsular system or the chemical structure of the polysaccharide produced."[28] It was

"some job, full of headaches and heartbreaks," but they persevered.[29] The presence of contaminating amounts of the enzyme capable of degrading DNA that remained during the isolation procedure, and which at the time went unrecognized, gave erratic results for the transformation experiments. "Many were the times, when we were ready to throw the whole thing out the window."[2]

The results of their findings were presented in a paper published in 1944 in the *Journal of Experimental Medicine,* which is a model of factual statement and careful analysis.[30] That the authors fully recognized the genetic significance of their work is clearly indicated by the opening statement of the introduction: "Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted as hereditary characters. Among microorganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific Types of Pneumococcus." They then went on to state that "the major interest has centered on attempts to isolate the active principle from crude bacterial extracts and to identify if possible its chemical nature or at least to characterize it sufficiently to place it in a general group of known chemical substances." There follow descriptions of their methods of measuring transforming activity and the preparation of active extracts, differing slightly but significantly from the techniques Alloway used.[23] They routinely included the heat killing of the cells at 65°C before extraction to inactivate the enzymes that could destroy the transforming factor. Another important innovation was the use of chloroform to precipitate and remove protein from the material after it had been precipitated as described by Alloway and then redissolved in salt solution.[31] The carbohydrate responsible for the immunological reaction was then removed by the addition of a purified specific enzyme known to destroy this substance, and the reaction was allowed to continue until the extremely sensitive immune response was no longer observable. The residual solution was then precipitated with alcohol and redissolved a total of four to five times. The result was an exteremely pure substance—none other than the genetic material.

The approach to identifying the chemical nature of the material was essentially a process of elimination. It was apparently not carbo-

Figure 7.4 Colin MacLeod (courtesy of *Cancer Research 35*, cover [1975]).

Figure 7.5 Maclyn McCarty (courtesy of *Cancer Research 35*, cover [1975]).

hydrate, as indicated by the enzymatic hydrolysis step used in the preparation, did not have the solubility property of fats in alcohol and ether, and was apparently not protein as shown by both the deproteinization procedure used and negative results of the sensitive chemical tests (Biuret and Millon) employed to detect protein. Also, pure enzymes that destroy proteins showed no effect on the transforming activity of the substance. This left the nucleic acids as the only potential major group of known chemical substances. While a weak positive response was found with a test (orcinol) for RNA, this may have been an artifact, for the application of a pure crystalline enzyme that rapidly cleaved RNA had no effect on the transforming properties. On the other hand, a strong positive response was observed with a test (Dische) for DNA, and chemical analysis of the pure substance gave an elemental composition very close to that expected for DNA. Assuming the tetranucleotide structure, for example, the amount of phosphorus in different preparations was 8.5 to 9.1 percent. Tests on the purified transforming material to determine its immunological specificity with antibodies for both the carbohydrate and protein components of pneumococcus were negative. These sensitive tests were a further indication of the absence of contaminants in these preparations. Finally, that the chemical and physical properties were in fact those of DNA was shown by comparison with known preparations of DNA (including a sample supplied by Alfred Mirsky, also at Rockefeller). A preparation of an enzyme, desoxyribonuclease, known to degrade DNA, completely destroyed transforming activity.[32] Studies in the ultracentrifuge gave a molecular weight of the active substance estimated at 500,000, and the UV absorption properties were characteristic of nucleic acids. Avery, MacLeod, and McCarty stated in their summary at the end of the paper: "The data obtained by chemical, enzymatic and serological analyses together with the results of performing studies by electrophoresis, ultracentrifugation and ultraviolet spectroscopy indicate that, within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid or serologically reactive polysaccharide and consists principally, if not solely, of a highly polymerized, viscous form of desoxyribonucleic acid."[30] They then gave their final conclusion: "The evidence presented supports the belief that a nucleic acid of the desoxyribose Type is the fundamental unit of the transforming principle of Pneumococcus Type III."

Several researchers questioned Avery and his associates' conclusions as a result of the prevalent belief in the genetic primacy of proteins. Alfred Mirsky, also working at the hospital of the Rockefeller Institute, has been mentioned by Hotchkiss[2] and Stent[33] as one of the chief questioners of the idea that DNA was the transforming substance. Mirsky's views at the time were expressed as follows:

Avery and his colleagues have shown decisively by inactivation experiments that desoxyribose nucleic acid is an essential part of the transforming agent, and if there actually is no protein in their preparation, it would be obvious that the agent consists of nothing but nucleic acid. This is a conclusion of the greatest interest in the study of the chemical basis of biological specificity, and it should therefore be scrutinized carefully. There can be little doubt in the mind of anyone who has prepared nucleic acid that traces of protein probably remain in even the best preparations. With the tests now available for detecting how much protein is present in a nucleic acid preparation, it is probable that as much as 1 or 2 percent of protein could be present in a preparation of "pure, protein-free" nucleic acid. One of the most sensitive direct tests for protein is the Millon reaction but in our experience a nucleic acid preparation containing as much as 5 percent of protein would give a negative Millon test. At present the best criterion for the purity of a nucleic acid preparation is its elementary composition and especially the nitrogen:phosphorus ratio. Presence of 2 percent of protein would increase this ratio, but only by an amount that is well within the range of variation found for the purest nucleic acid preparation. No experiment has yet been done which permits one to decide whether this protein actually is present in the purified transforming agent, and if so, whether it is essential for its activity, in other words, it is not yet known which the transforming agent is—a nucleic acid or a nucleoprotein. To claim more, would be going beyond the experimental evidence.[34]

Therefore, the question revolved around the possible presence of a minute trace of extremely active protein in the transforming preparations—this notwithstanding the fact that Avery and his colleagues had shown that a final concentration of one part of the purified substance in 600,000,000 was enough to bring about transformation.[30] Noting the relative amounts of DNA and/or undetected protein possibly present, Mirsky nevertheless concluded, "there is, accordingly, some doubt whether DNA is itself the transforming agent."[35]

Mirsky's criticism was influenced, at least in part, by his own

studies with Hans Ris.[36] They had treated chromosomes with various salt solutions, similar to the studies Miescher had carried out much earlier. Two fractions were obtained. The fraction that dissolved was essentially DNA and constituted 90 to 92 percent of the mass of the chromosome. The insoluble fraction still retained the appearance of the chromosome, yet it contained only about 2 percent of the DNA. This apparent structure was destroyed if the chromosomes were treated with proteolytic enzymes that degraded the protein. But Avery's work suggested that DNA, not protein, was the primary component. "Thus, Avery's experiments showing only DNA to be essential for transformation (and thereby the truly essential chemical component of the pneumococcus chromosome) must have come as a considerable surprise to Mirsky in view of his own findings."[13] Mirsky emphasized the variable proportions of the DNA component and concluded, "The form of the chromosome is due primarily to the protein thread of the residual chromosome [which is] the basis for the linear order of the genes."[36] This work was carried out on nuclear material of mammalian origin (thymus gland), which is certainly more complex than bacterial chromosomes, and it is now known that the protein (histone) components do play a significant role in determining the overall structure and degree of expression of genes. However, the net effect of this work at that time was to reduce the potential significance of DNA.

To answer such objections, a second paper by Maclyn McCarty, and Avery, "Effect of Deoxyribonuclease on the Biological Activity of the Transforming Substance," was published in 1946.[37] In this study, they utilized purified deoxyribonuclease and in the summary of the paper stated, "It has been shown that extremely minute amounts of purified preparations of desoxyribonuclease are capable of bringing about the complete and irreversible inactivation of the transforming substance of Pneumococcus Type III." They also published a third paper providing an even more efficient procedure for isolating the transforming substance from several types of pneumococcus, the innovation being the addition of a substance (citrate) to prevent action of the degradative enzymes present that could cleave the DNA.[38] Thus, in 1946, McCarty concluded "that the accumulated evidence has established beyond a reasonable doubt that the active substance responsible for transformation is a nucleic acid of the deoxyribose type."[39]

Rollin Hotchkiss had been at the Rockefeller Institute from 1935 to 1937. On returning from a year in Copenhagen in 1938, he asked Avery if he could work with him on transformation but was delayed from doing so until much later.[25] Eventually Avery asked him to help distinguish the protein and nucleic acid components in the preparations of transforming material. Hotchkiss was able to show that the proportions of the nucleic acid bases present differed from those in thymus DNA and that a trace of the amino acid glycine was produced by hydrolysis of the purine base adenine, which could account for all the so-called protein component (estimated at 0.2 percent of the nitrogen) found to be present in the transforming material.[40] These results were published in 1949 in the form of a symposium paper in French and consequently had little impact on the debate on the transforming substance. Hotchkiss realized he was "naive" not to have published this work in a more accessible form elsewhere until much later.[2]

Avery was influenced by P. A. Levene to believe that DNA was unlikely to possess the properties requisite of the genetic material (see chapter 4). This would have made his approach to determining the chemical nature of the transforming substance a matter more of elimination than of direct determination. Avery also had a typically cautious approach to research; he said, "It is lots of fun to blow bubbles, but it is wiser to prick them yourself before someone else tries to."[29] But even in 1936, as Hotchkiss reported, "Avery outlined to me that the transforming agent could hardly be carbohydrate, did not match very well with protein, and wistfully suggested that it might be a nucleic acid."[25] In an eloquent letter in March 1943 to his brother Roy, a microbiologist at Vanderbilt University Medical School, Avery described the results of elemental analysis of the pure transforming substance, which "conforms *very* closely to the theoretical values of pure deoxyribose nucleic acid (thymus) Type," and added, "Who would have guessed it?"[29] Yet he fully realized the implications of this result: "If we are right, and of course that is not yet proven, then it means that nucleic acids are not merely structurally important but functionally active substances in determining the biological activities and specific characteristics of cells"; and later, "sounds like a virus—may be a gene."[29] This letter may have been a vehicle to someone he trusted wherein he could dispense somewhat with his natural caution and "blow bubbles." Avery also confided his views to a young naval researcher, Alvin Coburn, in March 1943.

Coburn sent a note after this meeting acknowledging the "most inspiring experience that I have had in medicine," which provides some independent evidence of Avery's awareness of the significance of his discovery.[41] Coburn also related an amusing anecdote illustrating Avery's high degree of cautiousness, which occurred "during a drive from Manhattan to Long Island across the windswept Triborough Bridge. Dr. Kneeland was at the wheel beside his passenger, Avery, who looked down at the dashboard and saw the indicator at the number 80. Avery inquired: 'Don't you think that we are travelling a bit too fast, Dr. Kneeland?' The latter then also looked down and saw what was upsetting the ever-cautious Fess, and replied, 'Dr. Avery, according to the speedometer we are going a bit less than 40 miles an hour. That's the radio indicator you are looking at.' "[41]

Once the work was concluded and published, convincing others of the correctness of the conclusion that DNA was the transforming agent was not always easy. Similar transformation experiments in other bacterial systems proved very difficult, and only André Boivin using the *E. coli* system seems to have been initially successful.[42] Of course, there were those who questioned, and still question, the validity of these results and indeed the whole concept of genetic transformation by DNA. As Hotchkiss reported, "I was to face many a biologist or chemist who, with the authority of textbooks at his side, would demonstrate once again how you can get a large number by multiplying almost any tiny fraction by Avogadro's number," and this continued until the mid-1950s.[2] At what point did questioning Avery's results become unreasonable? Hotchkiss states, "If genetically active DNA was heresy, it was accompanied by evidence, and was opposed by ideas that had become ingrained with little evidence (heresy versus hearsay, almost)."[2,43] In 1952, Hershey and Chase published a study that seemed to convince most of those still skeptical that DNA was indeed the genetic material.[44] The experiment consisted of demonstrating that viruses that infected and replicated in bacterial cells did so by injecting their DNA into the cells.[45]

At the time of Avery's publication, there was a considerable body of evidence suggesting other interpretations of his experiment (see chapter 8). Several leading investigators, including T. S. Sonneborn and George Beadle, postulated that transformation was really the effect of the transforming preparation (DNA) on other genes that might themselves be composed of protein. One of the leading geneticists,

J. Lederberg, offered seven alternative explanations for the transformation experiments. Between 1947 and 1951, with the exception of Boivin, transformation studies proved difficult to repeat, gave erratic results, and were associated mainly with antigenic traits. Even the Avery group experienced difficulties. Many of these problems proved to be technical ones. Often serum samples, human and animal, were ineffective for transformation. This problem was eliminated once the factor in serum necessary for transformation was identified and isolated. For a while, it was though that deoxyribonuclease might have a mild ability to degrade protein in the transforming extracts. This complicated the interpretation of those experiments in which the loss of transforming activity was observed following treatment of the transforming extract with deoxyribonuclease. Further, the addition of the DNA to the cultures to produce transformation was inefficient compared to the much higher efficiencies of infection that geneticists working with phage were capable of obtaining. Finally, there was the negative evidence; traits such as resistance to certain drugs were initially found not to be transferable.

Two other pieces of information contributed to the resistance to the acceptance of Avery's work. One was Caspersson's fundamental studies of the ultraviolet absorbance of nucleic acids, which showed a lower nucleic acid content in the nucleus during telophase. Later, this was shown to result from a large accumulation of RNA in the cytoplasm, thus making the nuclear content appear to diminish by comparison. The decrease in nuclear content of nucleic acids was interpreted as showing the instability of DNA. Under these conditions, an unstable material such as DNA could not be the source of information for all the cellular activites. The other piece of evidence was the ratio of bases in DNA. Initially, most of the DNA analyzed for the ratio of different bases came from higher organisms, where the ratio is close to 1:1. The failure to observe differing base ratios, as was later found for bacterial DNA, further supported the premise that the composition of DNA did not vary sufficiently from organism to organism to account for the considerable differences in information content that would be expected.

Because of the initial resistance to the concept that DNA could be the genetic material, the work of Avery, MacLeod, and McCarty was not generally accepted immediately. By comparison, the findings of Griffith on transformation were quickly confirmed, although Avery

was reluctant to accept their implications. The proposal of Watson and Crick of a double-helical structure for DNA also was accorded rapid general acceptance.[46] One author has termed Avery's work the "undiscovered discovery."[19] Another has commented: "That he was not made a Nobel Laureate remains to this day a matter of painful surprise in many scientific circles."[17]

Considering the dearth of citation to the paper by Avery, MacLeod, and McCarty, Wyatt was led to the conclusion that "information . . . is unrecognized until it is transformed into knowledge."[47] However, an author's choice of literature citations is subjective and clearly inadequate as a means to determine the true impact of a given scientific contribution.[48,49] In the specific case of the work of Avery and his colleagues on transformation, the results were known to most of the individuals most capable of appreciating them. This included Salvador Luria[50] of the "phage group," Rollin Hotchkiss who remembered "serious discussions on the subject with Delbrück, Cohen and especially Hershey in the period 1949-51,"[51] Joseph Fruton who remembered "discussions at the lunch room of the Rockefeller Institute after Dr. Avery gave his paper,"[50] and discussions of this work at the Cold Spring Harbor symposium in 1946 and elsewhere.[2] Very little of these extensive personal contacts would be reflected in literature citations.

In analyzing the response to Avery and his colleagues' discovery and several other discoveries that were seemingly ignored by their contemporaries,[52] Gunther Stent defined the concept of "prematurity": "A discovery is premature if its implications cannot be connected by a series of simple logical steps to canonical, or generally accepted knowledge."[53] This provocative concept, while it may be a tautology,[50] appears to be similar to the view expressed by Arthur Koestler in *The Act of Creation,* that discovery is "the perceiving of a situation or idea in two self-consistent, but habitually incompatible, frames of reference," a process he termed *bisociation.*[54] Whatever the respective merits of these historiographical formulas, they do tend to ignore the unique aspects of situations in favor of their supposed similarities. Thus, they do not tell us why a particular discovery might be judged "premature." In the case of Avery and his colleagues' work on transformation by DNA, it is useful to remember that World War II was still in progress, that Avery was relatively old (sixty-seven years) when this work was published, that he had a reserved temperament,

and that several authorities questioned his conclusions in the light of the prevalent belief in the genetic primacy of proteins over DNA.

These factors presumably contributed to delaying the general acceptance of DNA as the transforming substance for eight years,[53] until the confirmatory results of Hershey and Chase in 1952.[44] Nevertheless, apart from Avery's coworkers, several others were active in this field in the intervening period, including R. Austrian, A. Boivin, S. Cohen, H. Ephrussi-Taylor, and S. Zamenhof. Many did in fact accept the implications of the results of Avery and his coworkers, including Erwin Chargaff.[55] For example, René Dubos wrote in 1945,

> Assuming that the substance which induces transformation is really a desoxyribonucleic acid, as the evidence strongly suggests, then nucleic acids of this type must be regarded not merely as structurally important, but as functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells; they possess a biological specificity, the chemical basis of which is as yet undetermined.[56]

For people who accepted the implications of this work, it could hardly be described as "premature."

It should also be noted that Griffith's original description of pneumococcal transformation was published in 1928 and that Alloway described the preparation of the pure cell-free transforming substance in 1933; but its identity was not determined by Avery and his coworkers until 1944. It appears from the *Scientific Reports of the Rockefeller Institute* that while Avery and MacLeod worked on pneumococcal transformation from 1934 to 1937, they dropped this project in favor of work on a specific protein found in the blood during infection.[57] This is confirmed by Hotchkiss: "In 1938, returning to his laboratory from a year in Denmark, I begged for an opportunity to work on transformation, but he [Avery] was anxious to further the work on blood proteins in acute infection, and asked me to wait saying 'we will get to that later.' "[25] In the *Scientific Reports* for 1940–1941, there is a report by MacLeod and Avery on capsular synthesis in pneumococci and in 1941–1942 by Avery and his associates on "bacterial virulence as manifested in infections produced by pneumococci." The first report on the "study of the chemical nature of the substance inducing transformation of specific Types of pneumococcus" by Avery and McCarty did not appear until the 1942–1943

Reports. This delay in tackling the basis of transformation is also confirmed by Avery himself in a letter to his brother, dated May 1943, in which he stated, "For the *past two years*, first with MacLeod and now with Dr. McCarty, I have been trying to find out what is the chemical nature of substances in the bacterial extract which produces this specific change" (emphasis added).[29] In fact, Avery published nothing from 1934 to 1941 and nothing on transformation from 1933 until 1944. Much has been made of the delay between the publication of the Avery work in 1944 and that of Hershey and Chase in 1952, yet no mention has been made of the longer delay in following up the studies on the chemical nature of the transforming agent by Alloway,[23] either in Avery's laboratory or elsewhere. It should also be noted that James Watson and Francis Crick's paper on the double-helical structure of DNA was published in 1953,[46] only one year after Hershey and Chase's work, and Watson was well aware before then of Avery and his coworkers' paper.[59]

For these reasons, one should perhaps consider Avery and his colleagues' discovery "belated," as were so many other aspects of the understanding of the true nature of DNA. Because a discovery can hardly be "premature" and "belated" simultaneously, it is preferable to discard such labels as being unnecessarily deterministic. There appears to be no reason to presume that the history of science is more of a science than history.